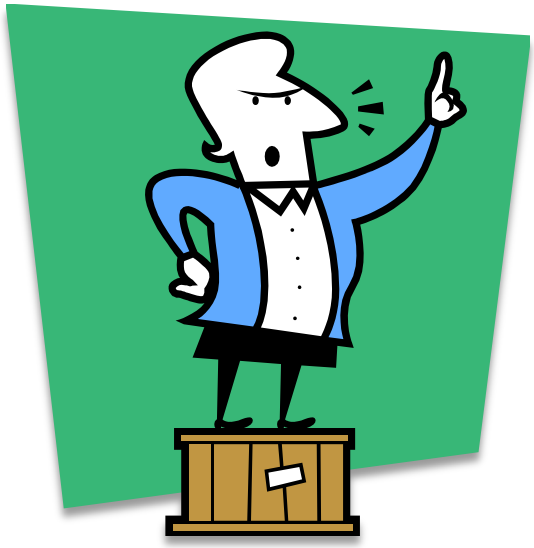
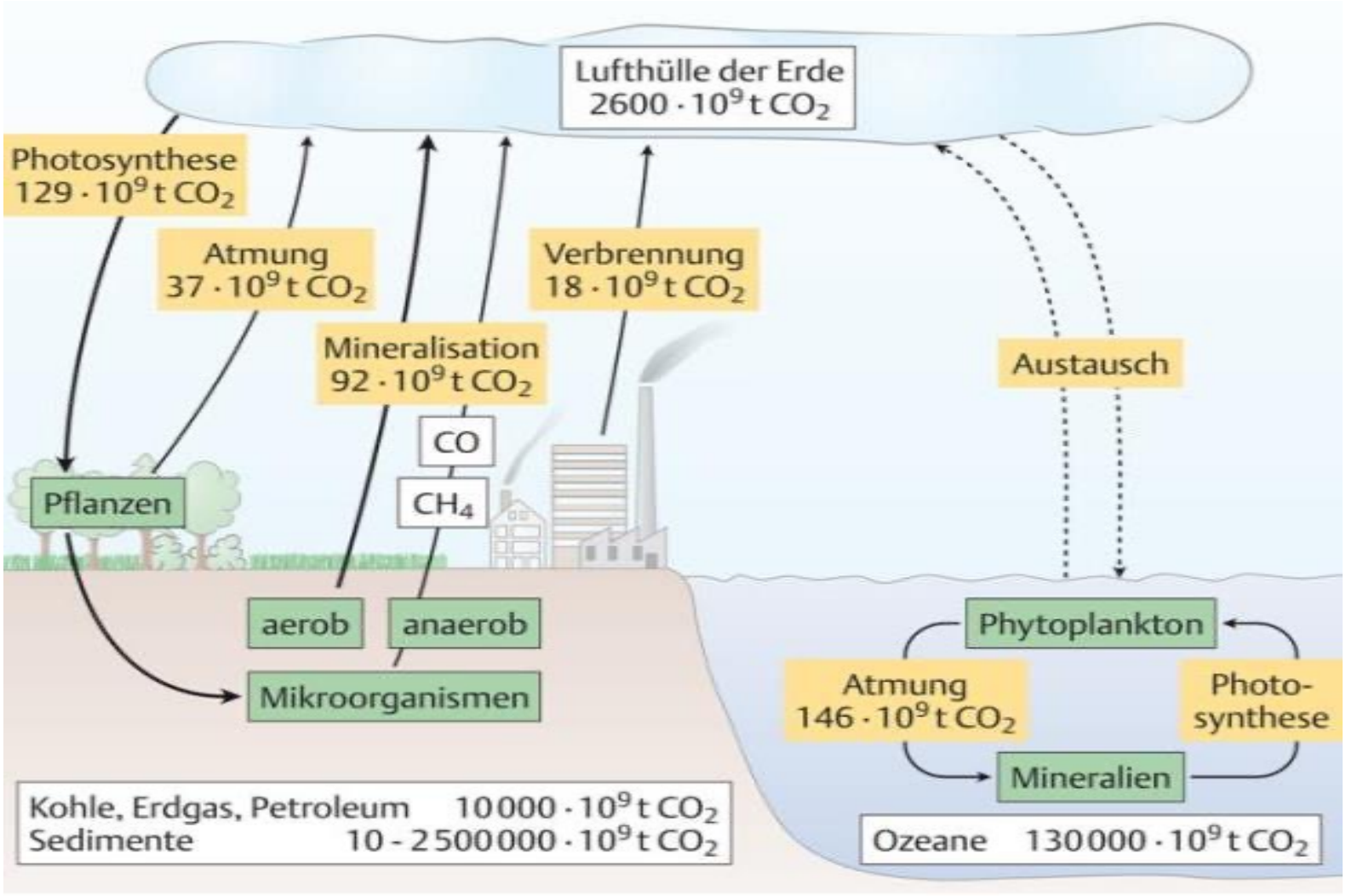
Thema der Vorlesung



Kohlenstoffkreislauf

Methanogenese
Methanoxidation
aerob und anaerob

Acetogenese
Homoacetogene MO

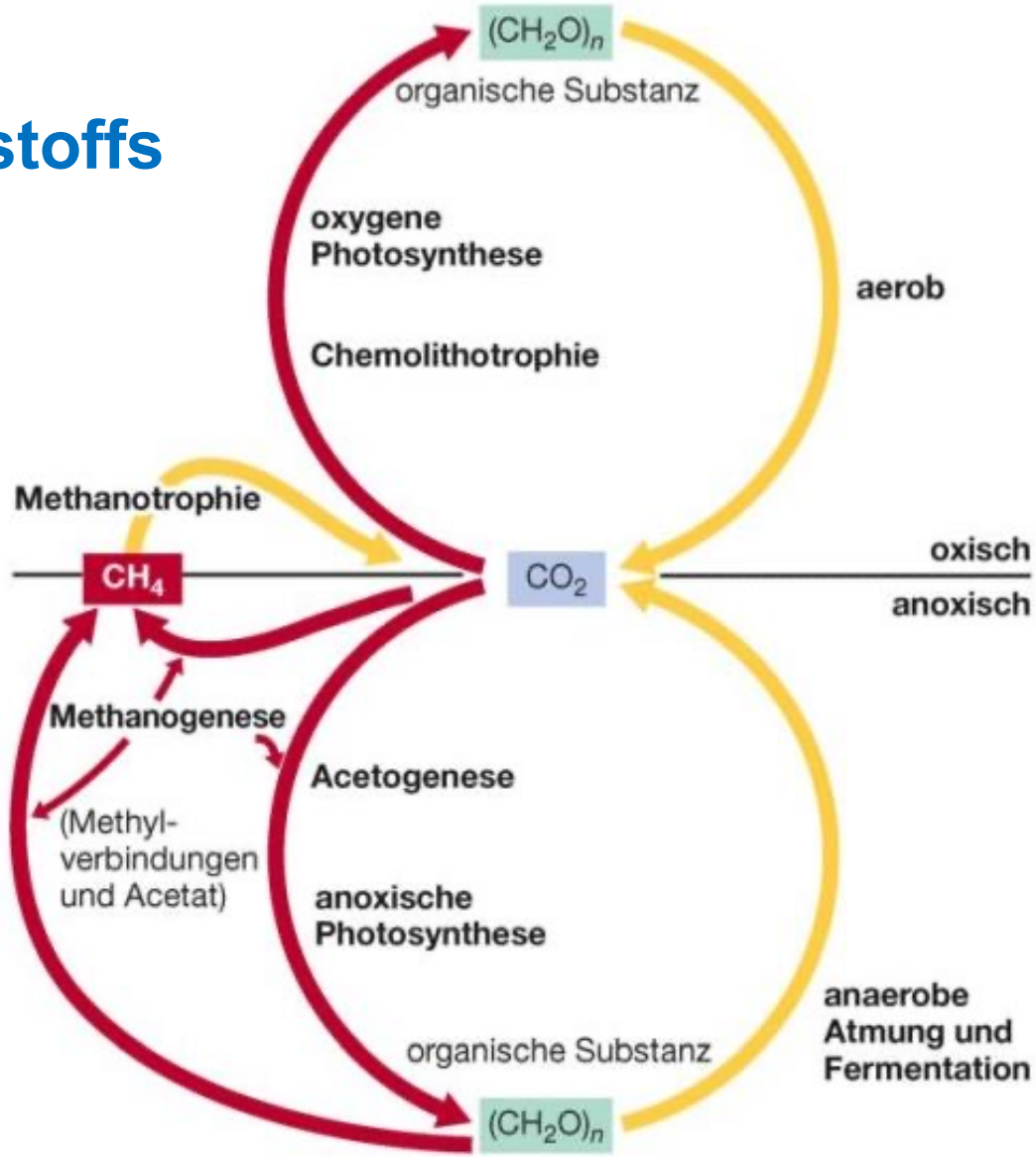


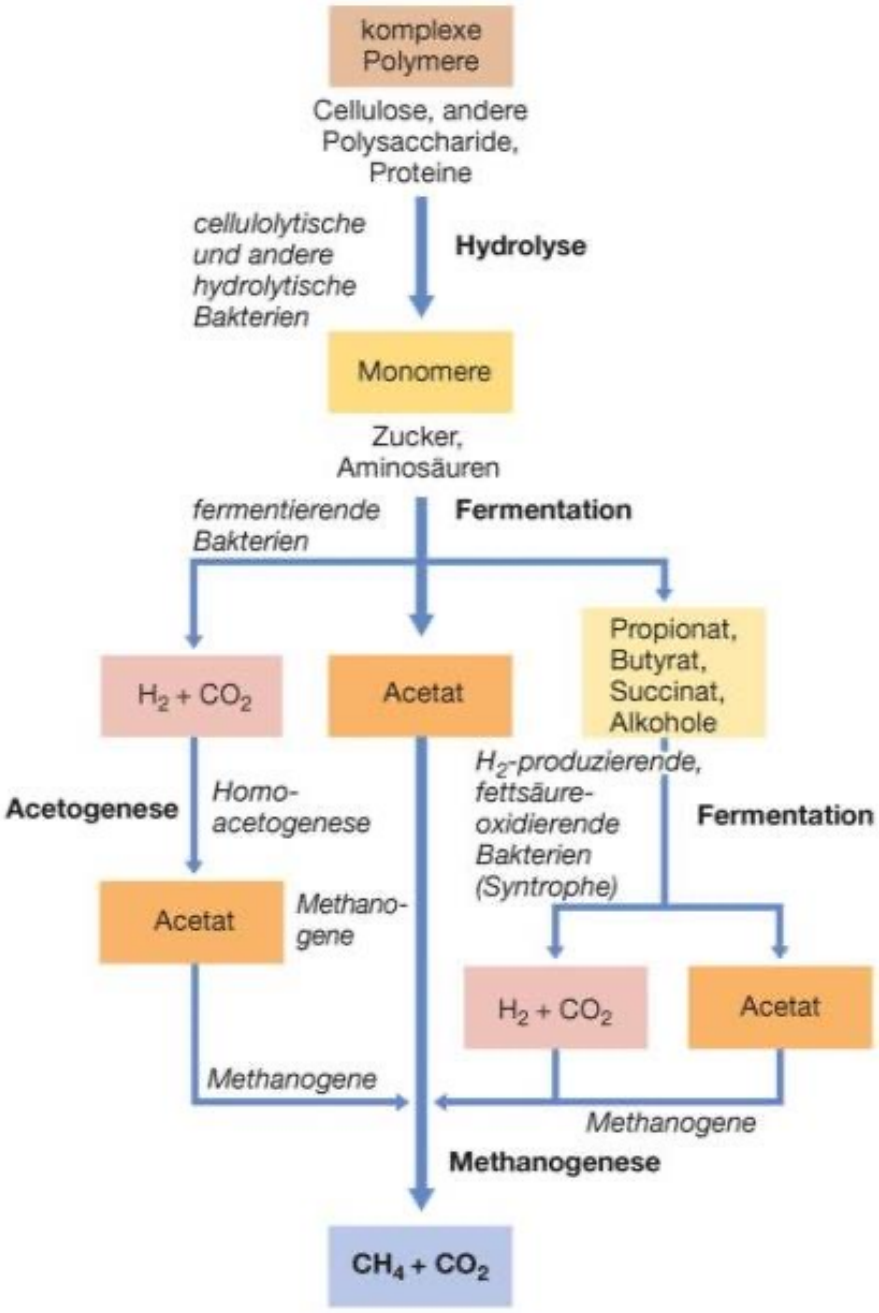
Georg Thieme Verlag, Stuttgart
Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage · 2006

Redoxzyklus des Kohlenstoffs

die Abbildung stellt die autotrophen Stoffwechselwege dar

die gelben Pfeile bezeichnen Oxidations-, die roten Reduktionsvorgänge

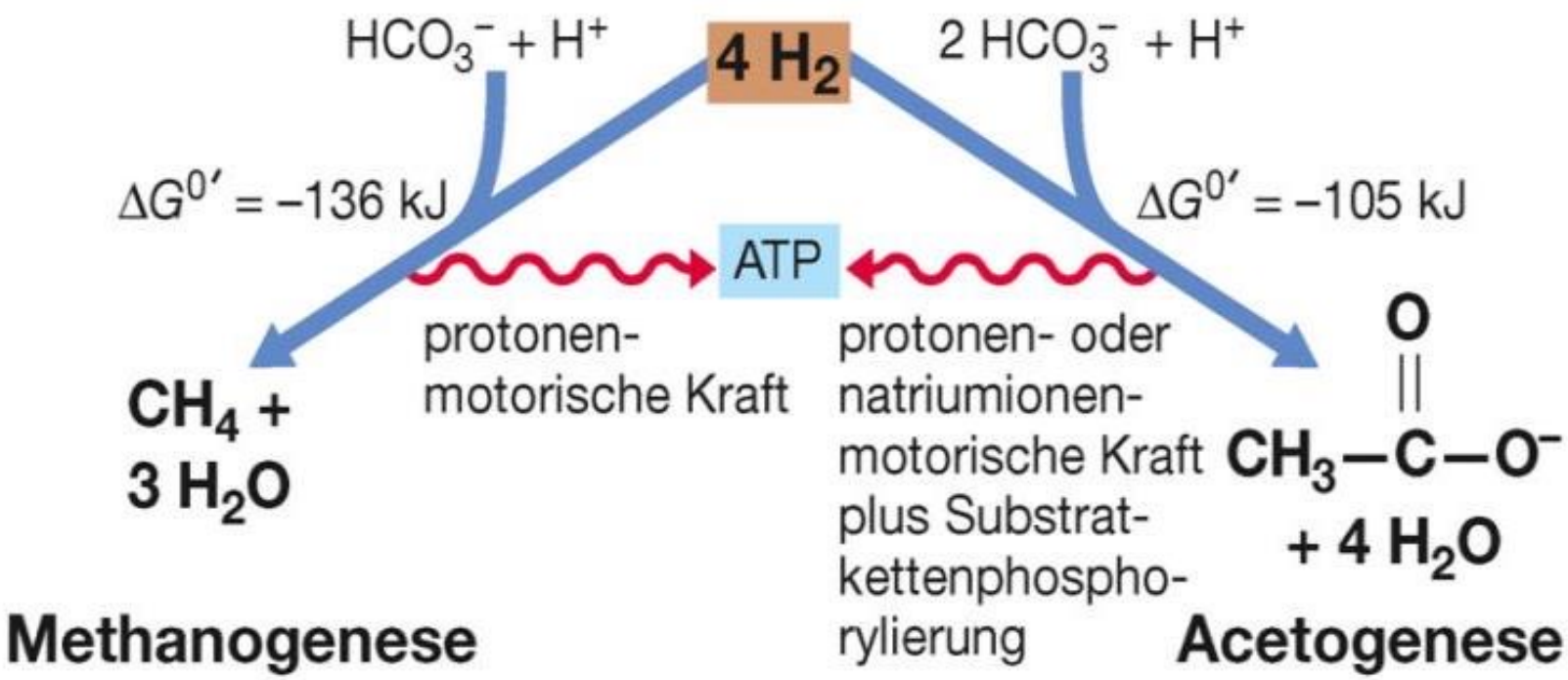




Anoxische Zersetzung

dargestellt ist schematisch der Gesamtprozess der anoxischen Zersetzung

verschiedene Gruppen fermentativer Anaerobier wirken bei der Umwandlung komplexer organischer Stoffe in Methan und Kohlendioxid kooperativ zusammen



Clostridien, Spirochaeten, Bacteriodetes, Acetobacterien

zwei Methoden, anaerob mit CO2 umzugehen!

2000

lobal

1000 1200 1400 1600

Year

Donald J. Trump
✓@realDonaldTrump
The concept of global warming was created by and for the Chinese in order to make U.S. manufacturing non-competitive.
8:15 PM - 6 Nov 2012

Methan wird freigesetzt aus Sumpfgebieten, Reisanbau, Tierzucht, fossilen Lagerstätten

schwimmendes Methanhydrat auf Meeresoberfläche

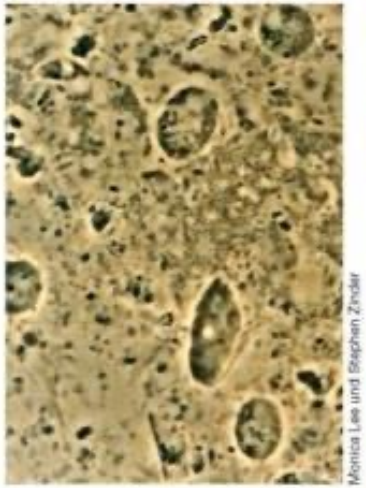
Morphologie geprägt durch Fluid- und Gasflüsse sowie Bildung, Aufschwimmen und Zersetzung von Gashydraten

Treibhausgas	**Anno 1750**	**Anno 1998**
Kohlendioxid CO_2	280 ppm	365 ppm
Methan CH_4	700 ppb	1745 ppb
Lachgas N_2O	270 ppb	314 ppb
troposph. Ozon* O_3	10 ppb	30-40 ppb
Freon-11 $CFCl_3$	0	268 ppt
Freon-12 CF_2Cl_2	0	533 ppt

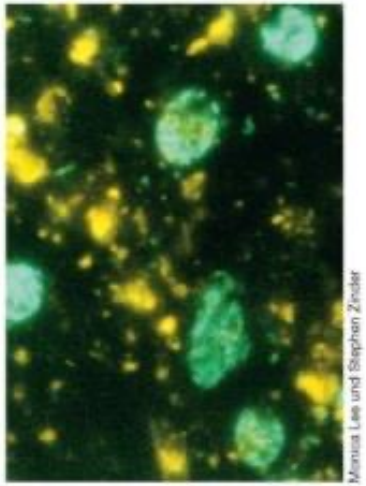
Wie viel Methan produziert eine Kuh pro Stunde?

http://www.youtube.com/watch?v=-X83sMnoy-U&noredirect=1

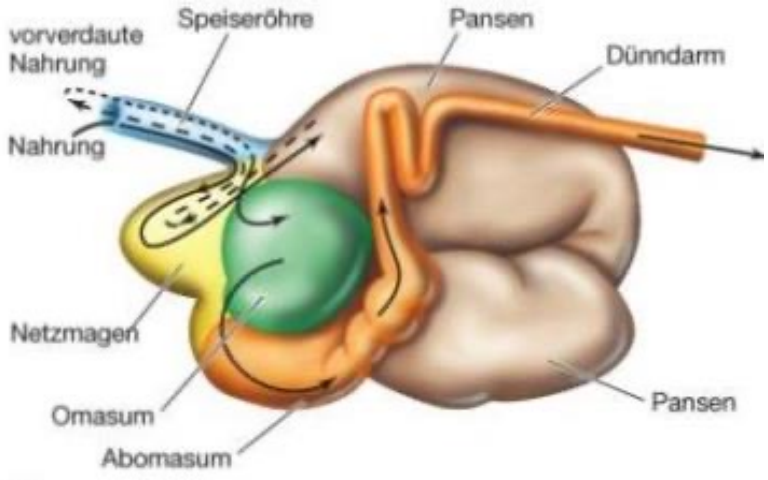
(a)



(b)



(c)



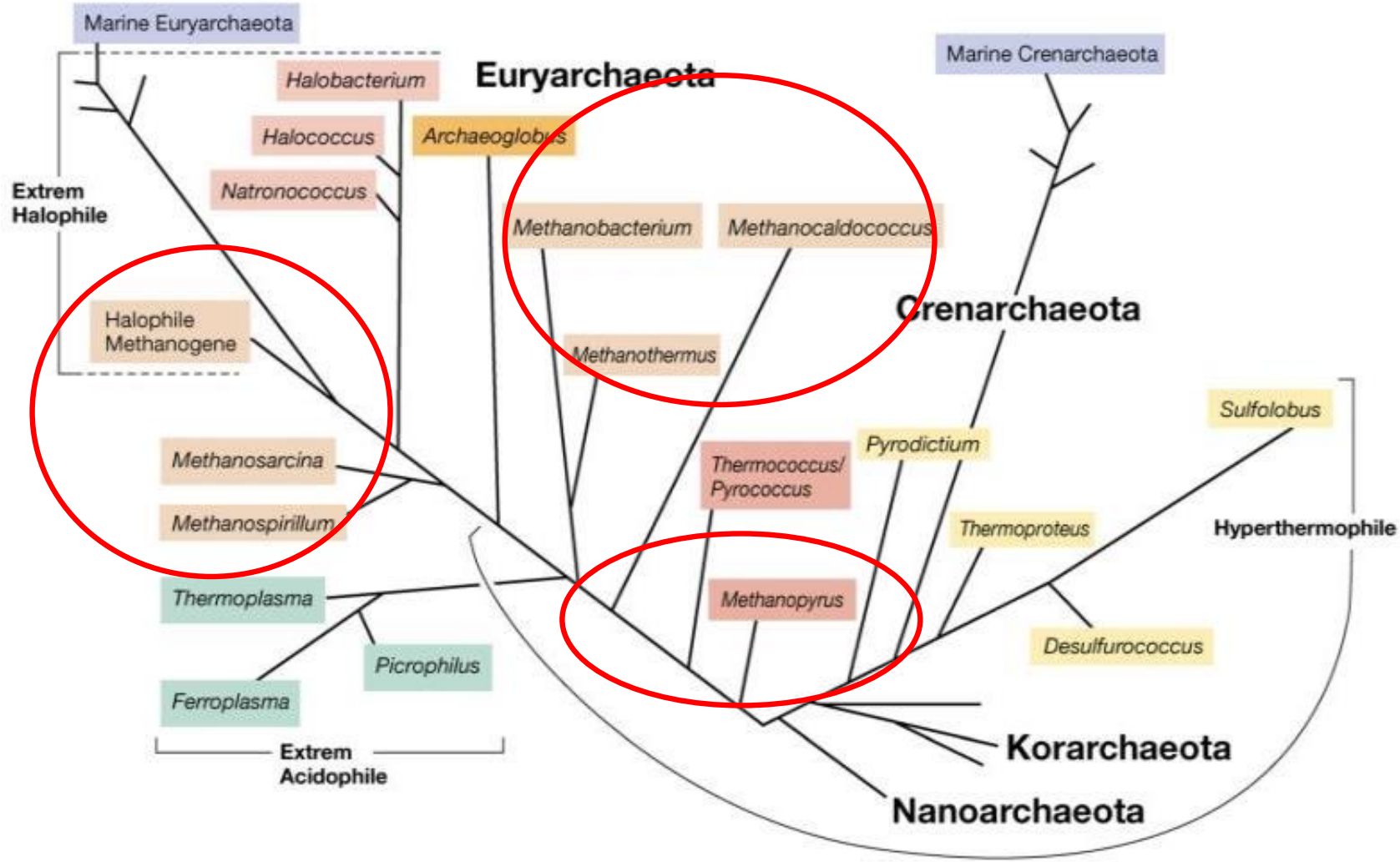
(a)

(b)

Termiten und der Intestinaltrakt von Wiederkäuern sind wichtige Habitate für **Methanogene**

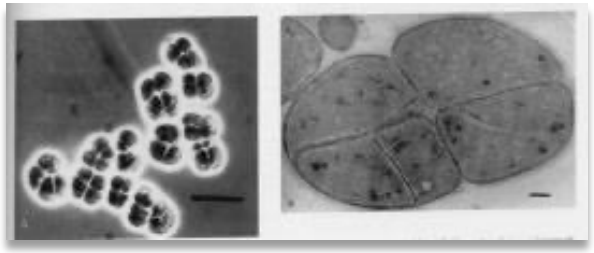
Habitate der Methanogenen

Tiefseesedimente
aquatische Sedimente allgemein
Kläranlagen (Faultürme)
Reisfelder (Nassreisanbau)
Permafrostböden
Intestinaltrakte von Säugetieren, Insekten etc.


Marine Euryarchaeota
Halobacterium
Euryarchaeota
Halococcus
Archaeoglobus
Natronococcus
Extrem
Halophile
Methanobacterium
Methanocaldococcus
Halophile
Methanogene
Methanothermus
Crenarchaeota
Marine Crenarchaeota
Sulfolobus
Pyrodictium
Methanosarcina
Thermococcus/
Pyrococcus
Methanospirillum
Thermoproteus
Hyperthermophile
Thermoplasma
Methanopyrus
Desulfurococcus
Picrophilus
Ferroplasma
Extrem
Acidophile
Korarchaeota
Nanoarchaeota

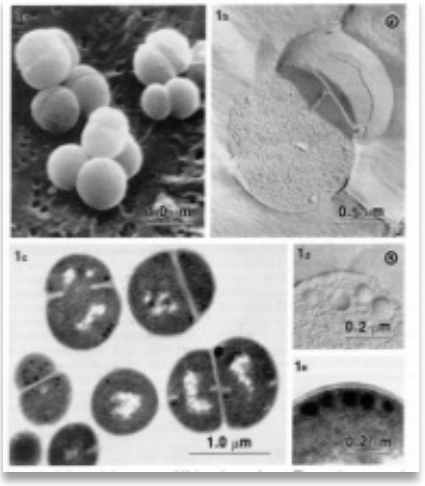
es gibt drei verschiedene Gruppen von **Methanogenen**, die anhand ihrer Substrate unterschieden werden:

- **Hydrogenotrophe Methanogene:** Die allermeisten Methanogene wachsen auf H_2 und CO_2



Ms. barkeri

- **Methylotrophe Methanogene:** Methylierte Verbindungen, besonders Methanol und Methylamine werden umgesetzt. Organismen sind in den allermeisten Fällen Mitglieder der Familie Methanosarcinaceae



Methanosphaera stadtmanae

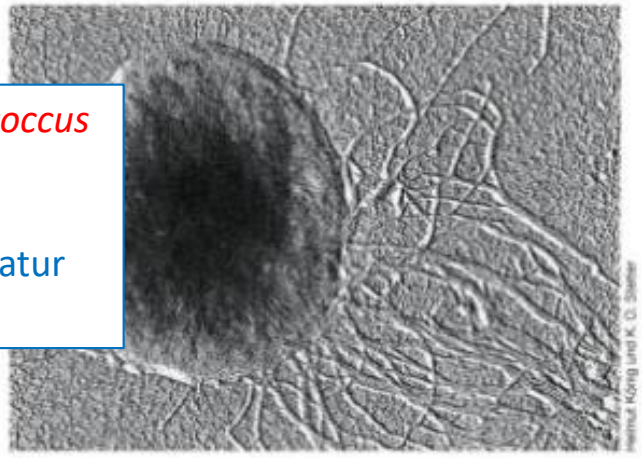
- **Acitoclastische Methanogene:** Acetat wird umgesetzt Nur die Gattungen *Methanosarcina und Methanothrix* (Methanoseta) sind dazu in der Lage

Steckbrief Methanogene

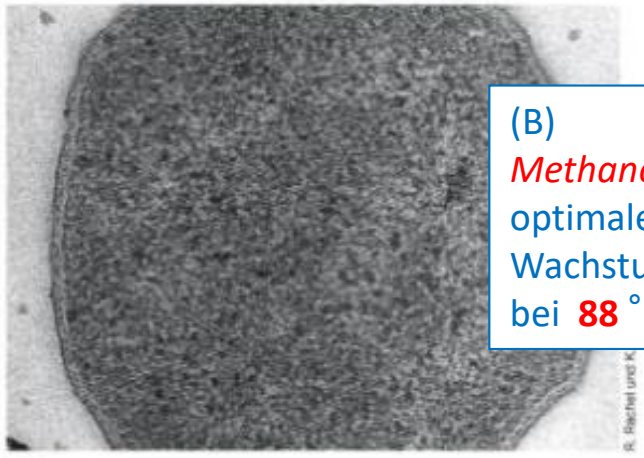
- Methanogene können in Süßwasser bis hin zu hypersalinen Habitaten gefunden werden, z.B. wächst *Methanohalophilus mahii* in bis zu 3M NaCl
- alle Methanogene sind Archaeen
- verwenden nur eine sehr kleine Gruppe von Substraten für die Methanbildung
- wachsende Methanogene sind strikte Anaerobier
- benötigen Na+ im Medium
- werden bei Temperaturen zwischen 2°C und 100°C gefunden
- die meisten Methanogenen wachsen bei neutralem pH, nur wenige Isolate sind moderat acidophil oder moderat alkaliphil

Hyperthermophile und thermophile Methanogene

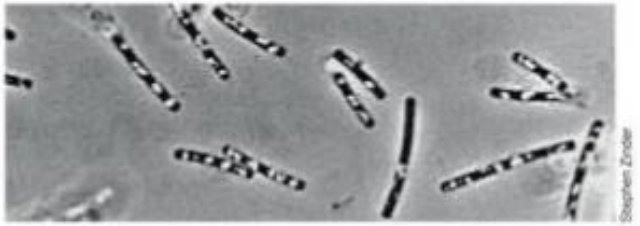
(A) *Methanocaldococcus jannaschii* optimale Wachstumstemperatur bei **85** ° C



(B) *Methanotorris igneus* optimale Wachstumstemperatur bei **88** ° C



(C) *Methanothermus fervidus* optimale Wachstumstemperatur bei **88** ° C



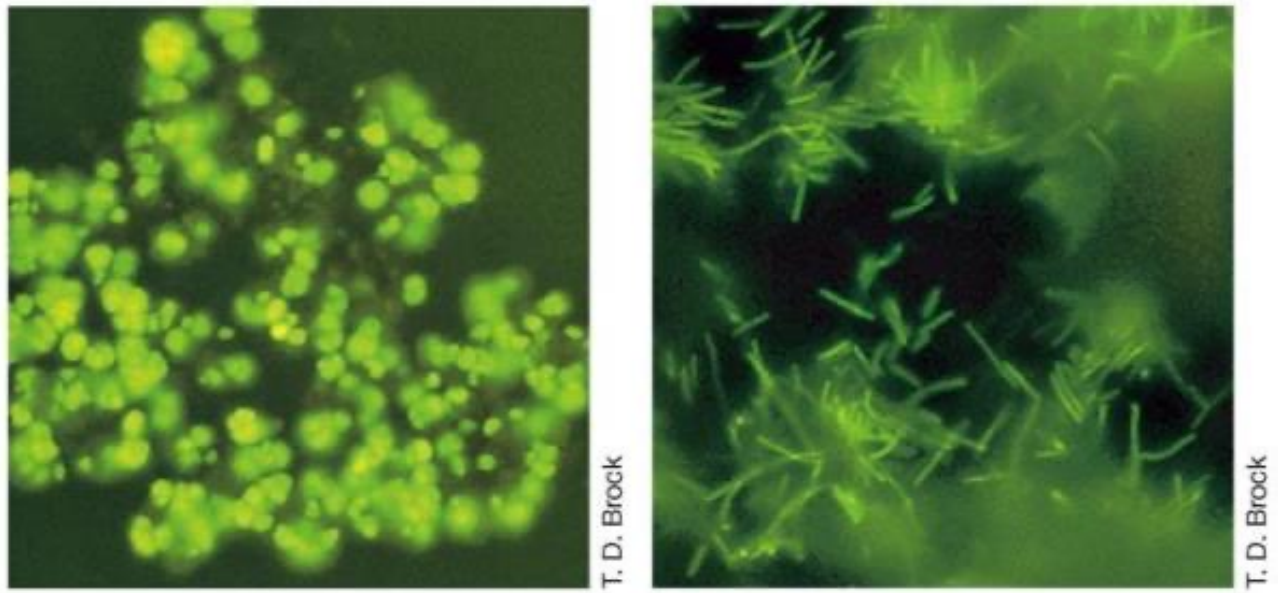
(D) *Methanosaeta thermophila* optimale Wachstumstemperatur bei **60** ° C

Genomgrößen der Methanogenen

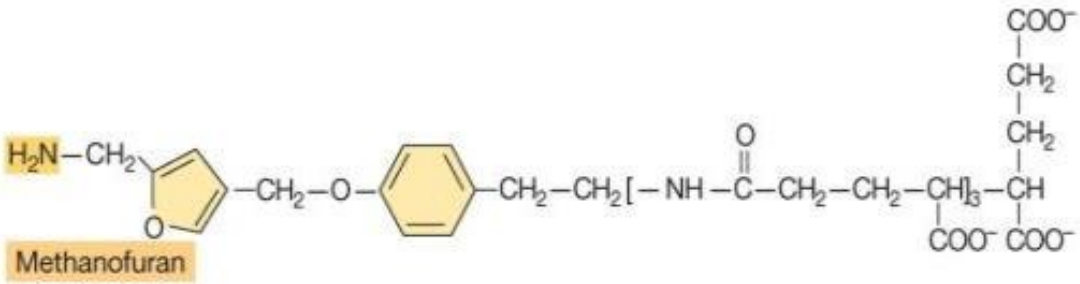
Organism	GenBank accession no.	Genome length (bp)	G+C content (%)	No. of features	% of genome with features	Mean no. of nt/feature
M. acetivorans	NC_003552	5,751,494	42.7	4,540	74	939
M. mazei	NC_003901	4,096,345	41.5	3,371	75	912
M. barkeri Fusaro	NC_007355	4,837,408	39.2	3,680	70	921
		4,873,766	39.2	3,698	69	922

Maeder et al., Journal of Bacteriology, 2006, p. 7922-7931

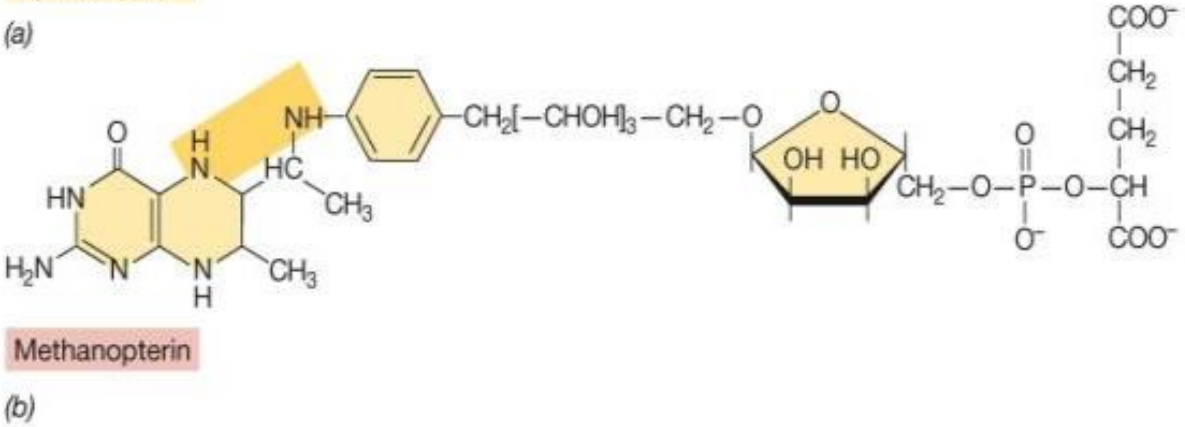
Methanogene fluoreszieren durch das Coenzym F_{420} **grün**



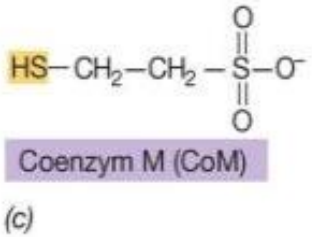
(a) Autofluoreszenz von Zellen des Methanogenen *Methanosarcina barkeri*, die auf die Anwesenheit des einzigartigen Elektronenüberträgers F_{420} zurückgeht.



Methanofuran ist am ersten Schritt beteiligt
initiale $C0_2$-Bindung über NH_2-Furanring

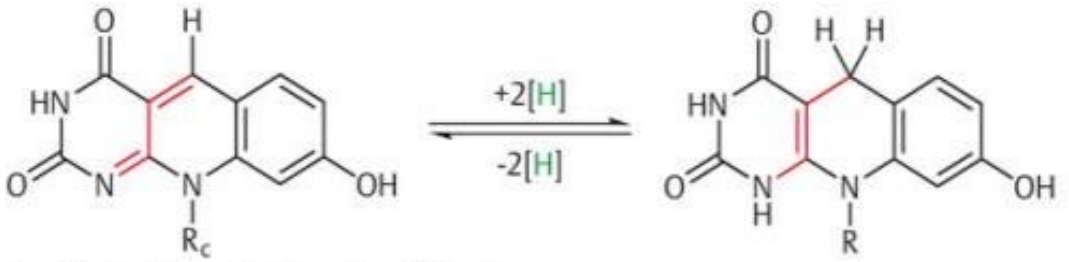


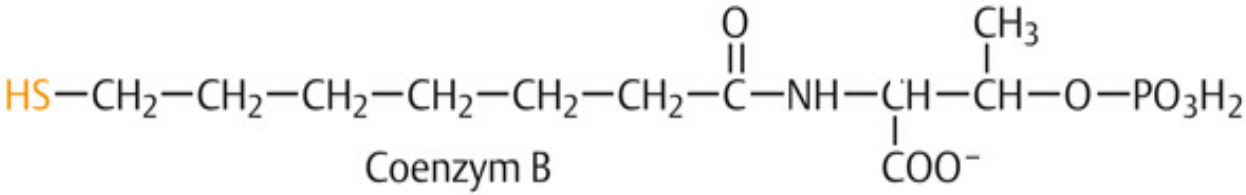
Methanopterin ist ein intermediärer C1-Überträger; Folsäurederivat ähnlich wie Vitamin B1



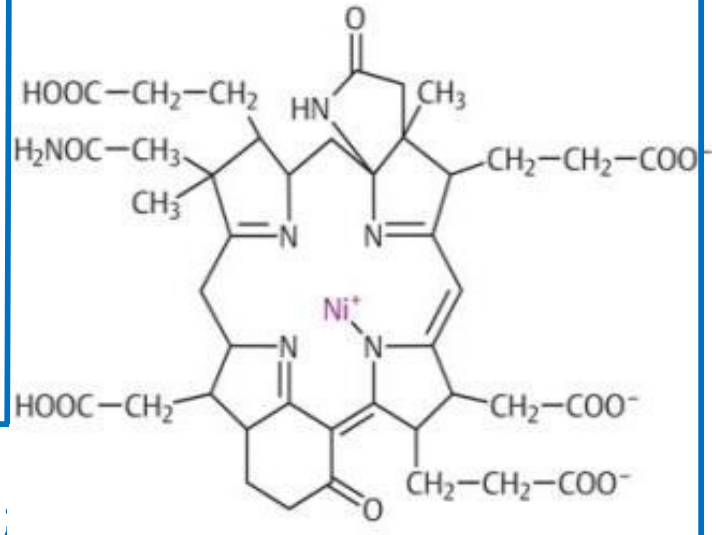
CoM ist am letzten Schritt der Methanogenese beteiligt; Überführung von gebundenem Methyl- zu freiem Methan

F 420 ist ein **Flavinderivat**;
es dient als Elektronendonator
die oxidierte Form absorbiert
Licht bei 420 nm, grüne Fluoreszens





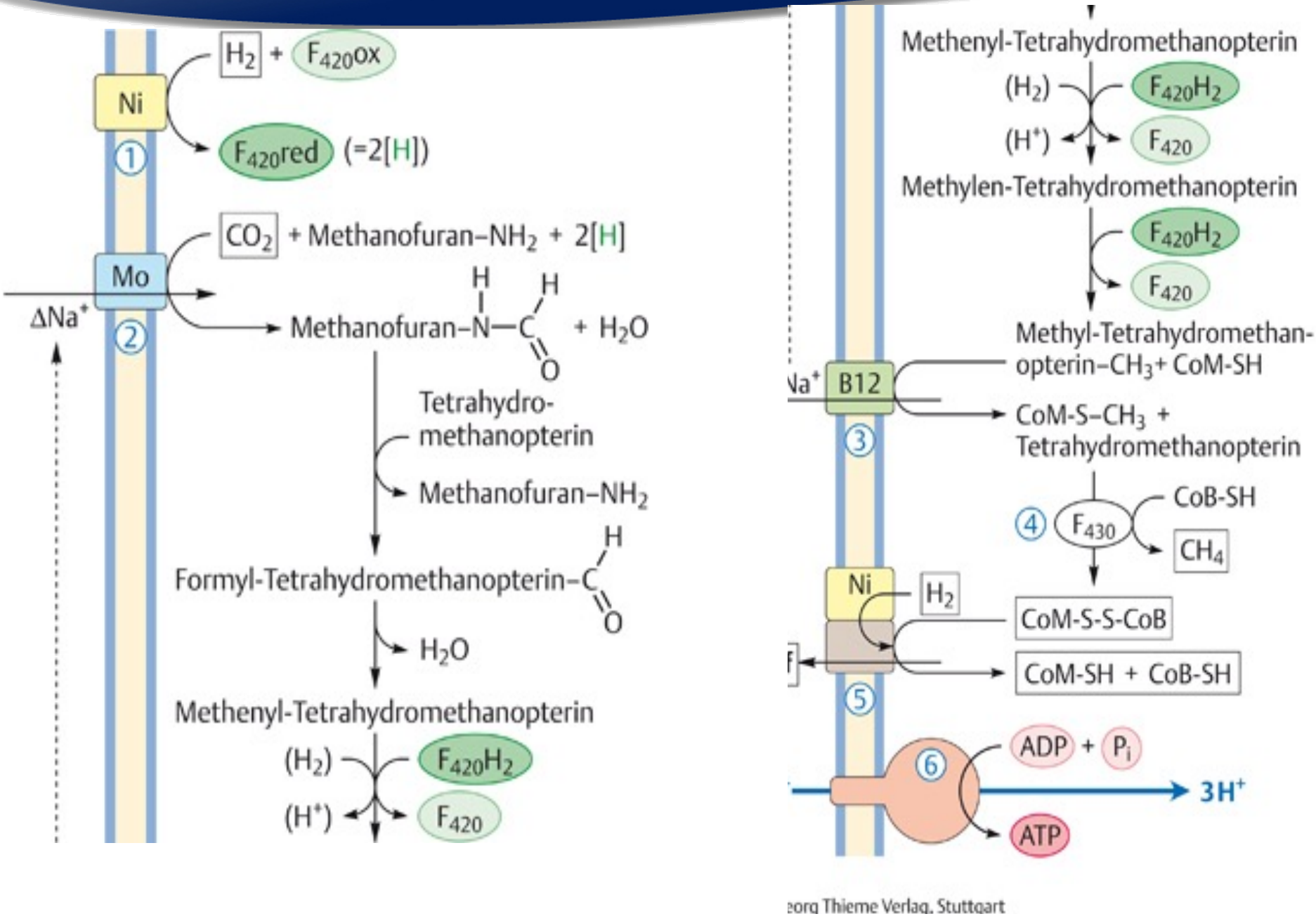
CoB ist ein **Elektronendonator**,
ähnelt dem Panthotenat;
letzer Schritt der Methanogenese



Faktor F_{430}

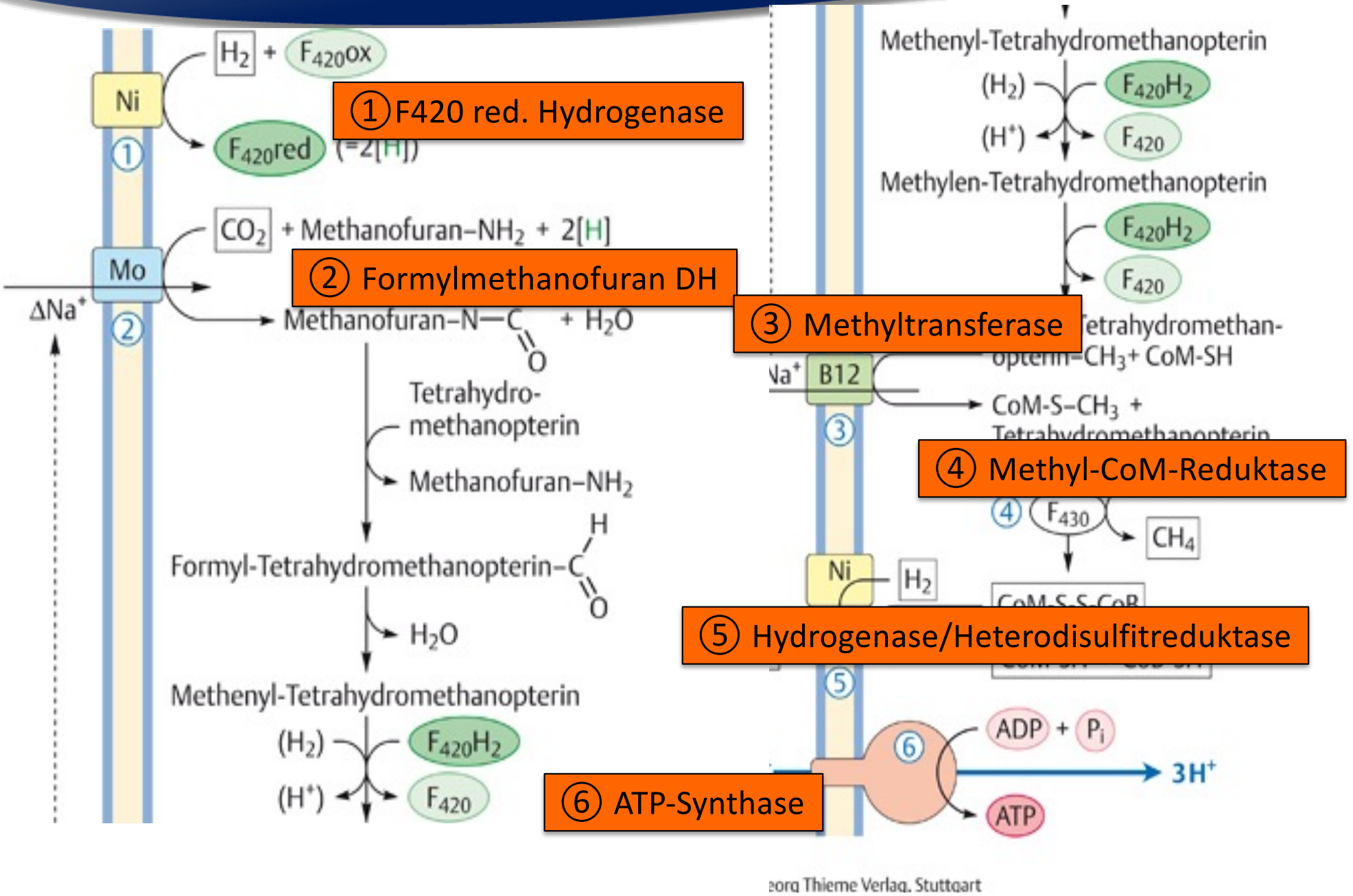
Faktor 430 ist am letzten Schritt beteiligt;
kein C1-Transfer; Ni-Tetrapyrrol-Komplex

CH_4 aus H_2/CO_2

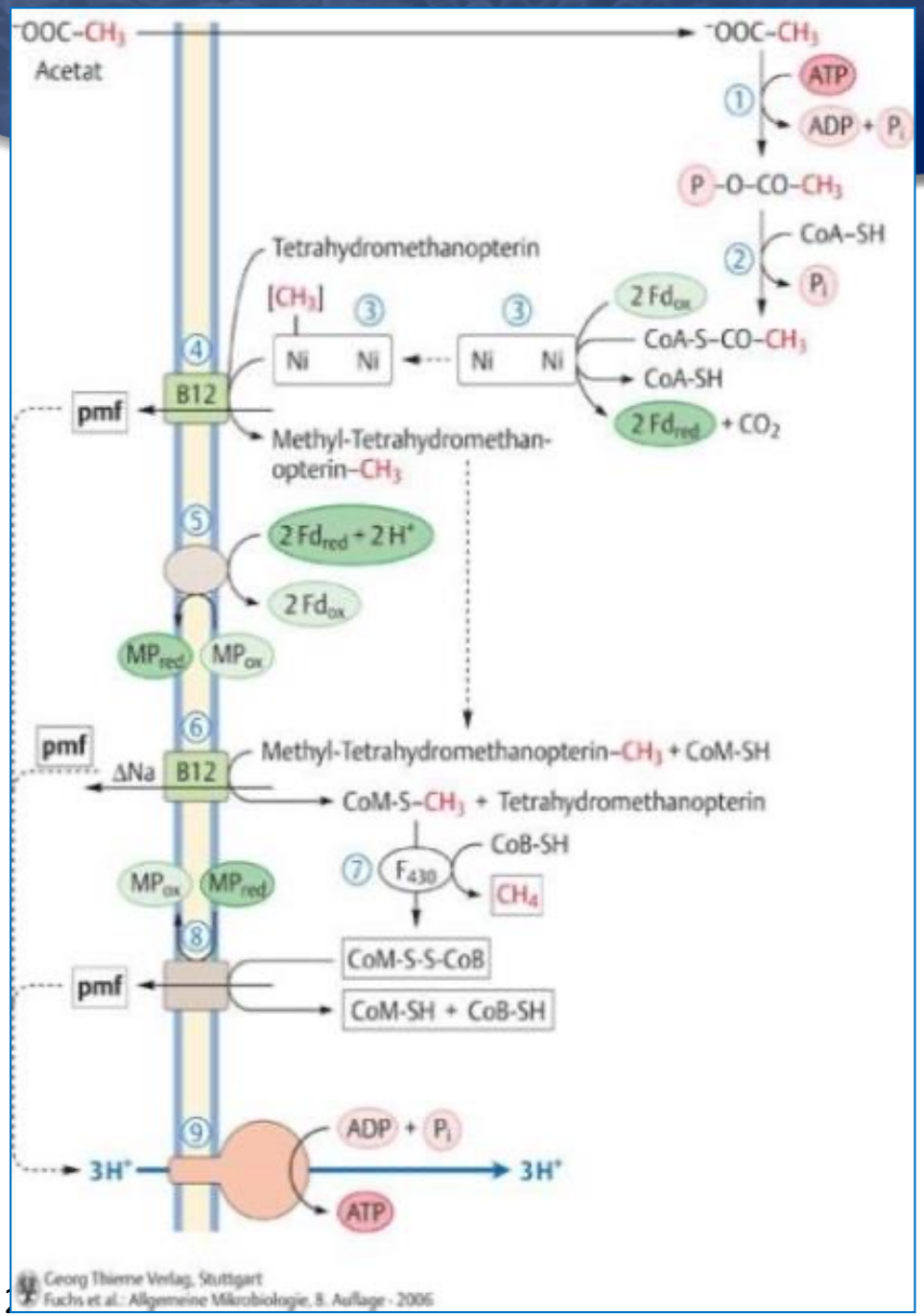


eorg Thieme Verlag, Stuttgart

CH_4 aus H_2/CO_2



eorg Thieme Verlag, Stuttgart



CH_4 aus Acetat

1. Acetatkinase
2. Phsophostransacetylase
3. CO-DH
4. Methyltransferase
5. Ferredoxin-Methanophenanzin Oxidoredukatse
6. Methlytransferase
7. Methyl-CoM-Reduktase
8. Hetreodislfidredukatse
9. ATP-Synthase
10. ....

Energetik der **Methanogenese**

$CO_2 + 4H_2 \rightarrow CH_4 + 2\ H_2O$ $\quad$ $G^{o\prime}$: -130 kJ/mol CH_4

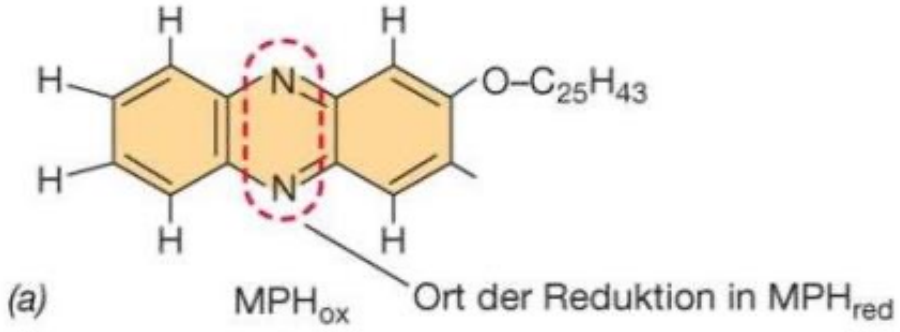
Energetik der **Methanogenese**

$CO_2 + 4H_2 \rightarrow CH_4 + 2\ H_2O$ -130 kJ/mol CH4

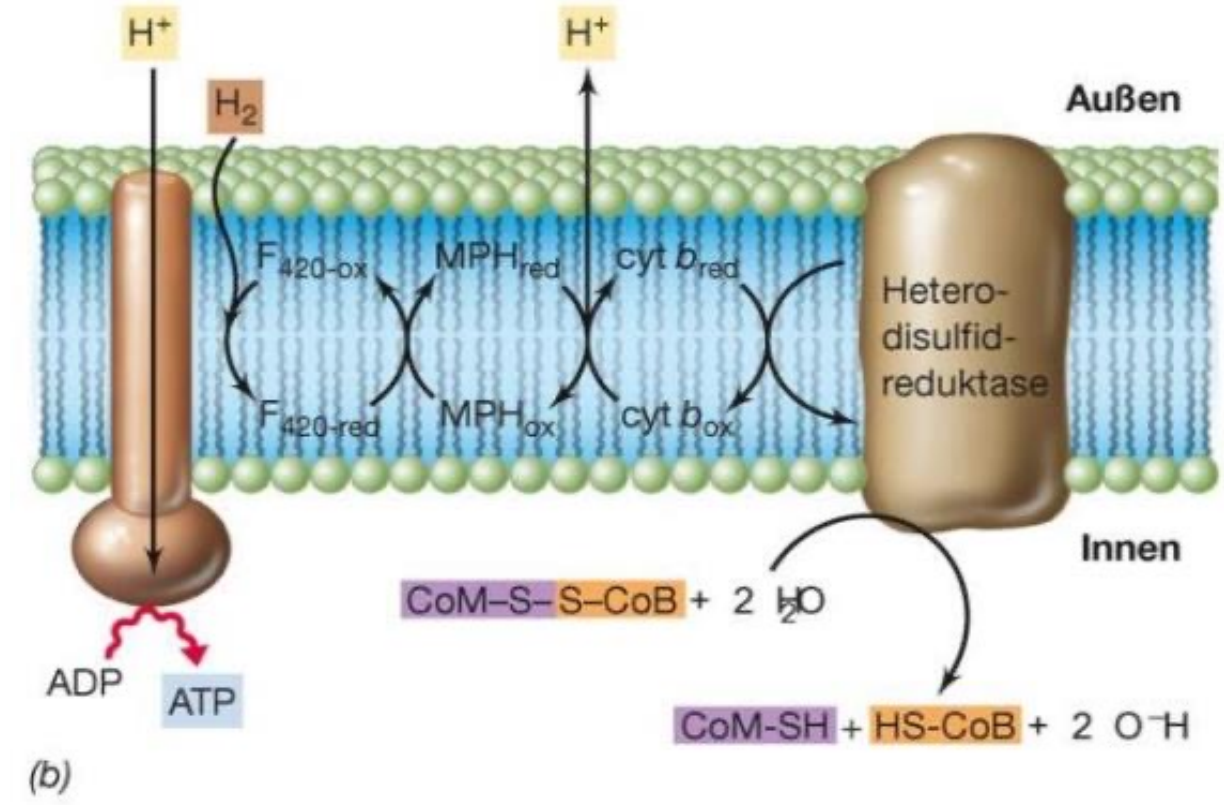
$4\ (CH_3)_3NH^+ + 6\ H2O \rightarrow 9\ CH_4 + 3\ CO_2 + NH_4^+$ -76 kJ/mol CH4

$2\ (CH_3)_2S + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 2\ H_2S$ - 49 kJ/mol CH4

$CH_3COO^- \rightarrow CH4 + CO_2$ - 36 kJ/mol CH4



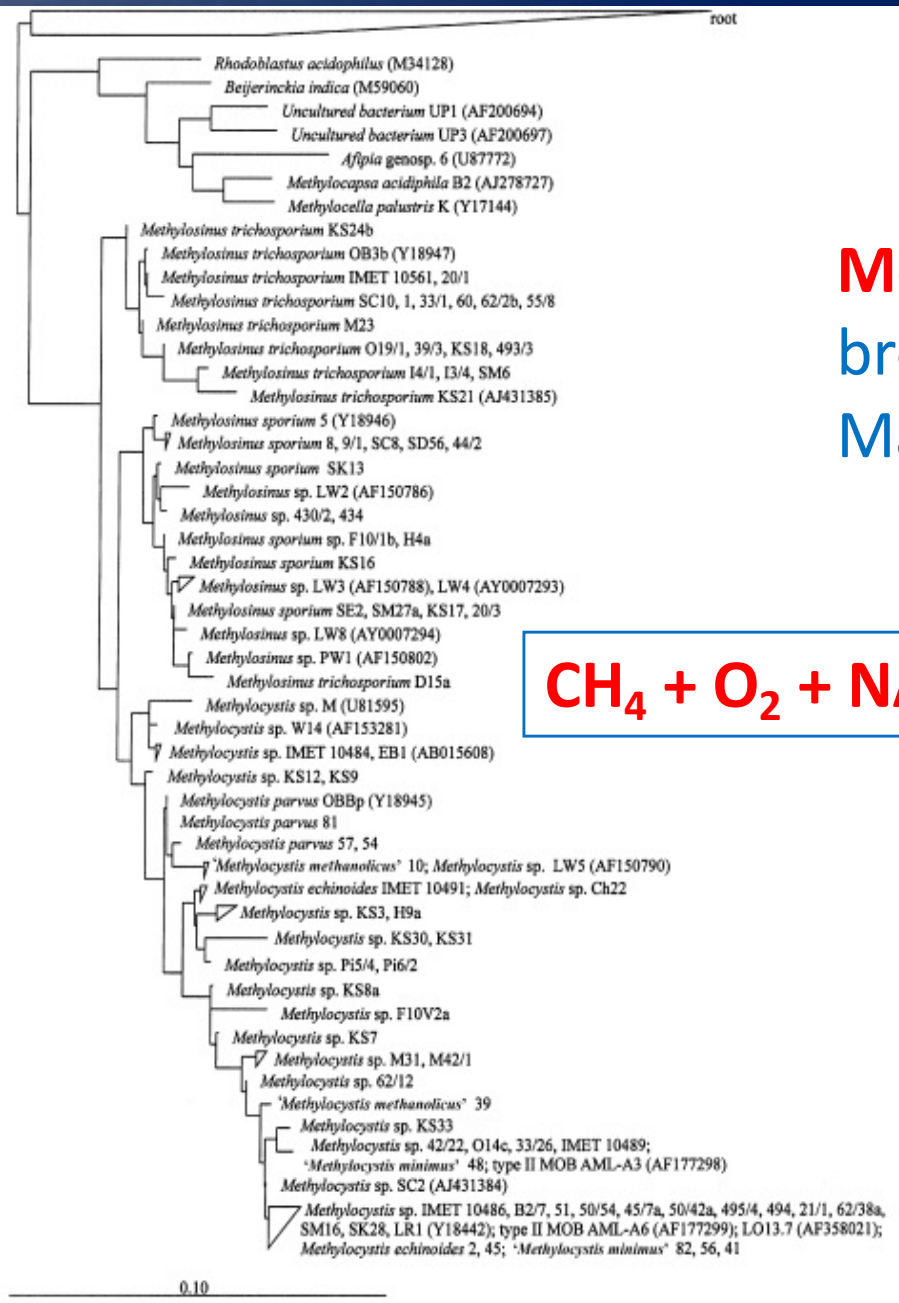
Energiespeicherung bei der Methanogenese



Und wie verschwindet das **Methan** wieder?

Oxidation des Methans

- aerob
- anaerob


root
Rhodoblastus acidophilus (M34128)
Beijerinckia indica (M59060)
Uncultured bacterium UP1 (AF200694)
Uncultured bacterium UP3 (AF200697)
Afipia genosp. 6 (U87772)
Methylocapsa acidiphila B2 (AJ278727)
Methylocella palustris K (Y17144)
Methylosinus trichosporium KS24b
Methylosinus trichosporium OB3b (Y18947)
Methylosinus trichosporium IMET 10561, 20/1
Methylosinus trichosporium SC10, 1, 33/1, 60, 62/2b, 55/8
Methylosinus trichosporium M23
Methylosinus trichosporium O19/1, 39/3, KS18, 493/3
Methylosinus trichosporium I4/1, I3/4, SM6
Methylosinus trichosporium KS21 (AJ431385)
Methylosinus sporium 5 (Y18946)
Methylosinus sporium 8, 9/1, SC8, SD56, 44/2
Methylosinus sporium SK13
Methylosinus sp. LW2 (AF150786)
Methylosinus sp. 430/2, 434
Methylosinus sporium sp. F10/1b, H4a
Methylosinus sporium KS16
Methylosinus sp. LW3 (AF150788), LW4 (AY0007293)
Methylosinus sporium SE2, SM27a, KS17, 20/3
Methylosinus sp. LW8 (AY0007294)
Methylosinus sp. PW1 (AF150802)
Methylosinus trichosporium D15a
Methylocystis sp. M (U81595)
Methylocystis sp. W14 (AF153281)
Methylocystis sp. IMET 10484, EB1 (AB015608)
Methylocystis sp. KS12, KS9
Methylocystis parvus OBBp (Y18945)
Methylocystis parvus 81
Methylocystis parvus 57, 54
'Methylocystis methanolicus' 10; Methylocystis sp. LW5 (AF150790)
Methylocystis echinoides IMET 10491; Methylocystis sp. Ch22
Methylocystis sp. KS3, H9a
Methylocystis sp. KS30, KS31
Methylocystis sp. Pi5/4, Pi6/2
Methylocystis sp. KS8a
Methylocystis sp. F10V2a
Methylocystis sp. KS7
Methylocystis sp. M31, M42/1
Methylocystis sp. 62/12
'Methylocystis methanolicus' 39
Methylocystis sp. KS33
Methylocystis sp. 42/22, O14c, 33/26, IMET 10489;
'Methylocystis minimus' 48; type II MOB AML-A3 (AF177298)
Methylocystis sp. SC2 (AJ431384)
Methylocystis sp. IMET 10486, B2/7, 51, 50/54, 45/7a, 50/42a, 495/4, 494, 21/1, 62/38a,
SM16, SK28, LR1 (Y18442); type II MOB AML-A6 (AF177299); LO13.7 (AF358021);
Methylocystis echinoides 2, 45; 'Methylocystis minimus' 82, 56, 41
0.10

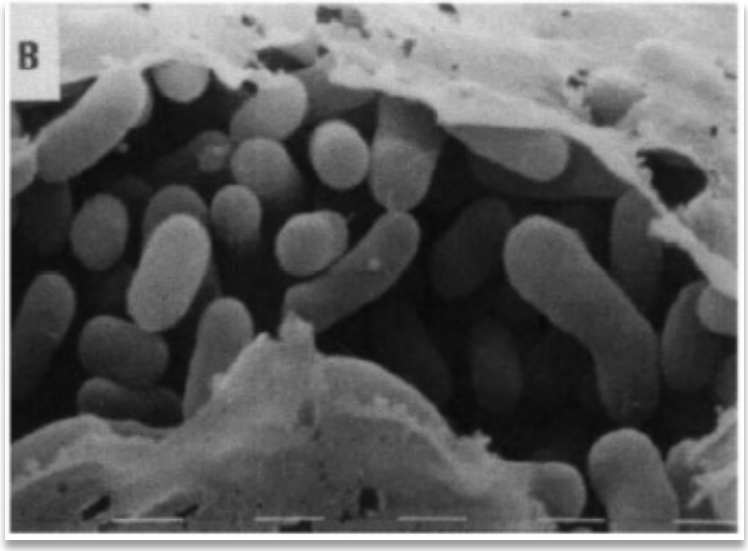
Methylotrophe Mikroorganismen

breit gefächerte Gruppe von MO

Man unterscheidet Typ I und Typ II

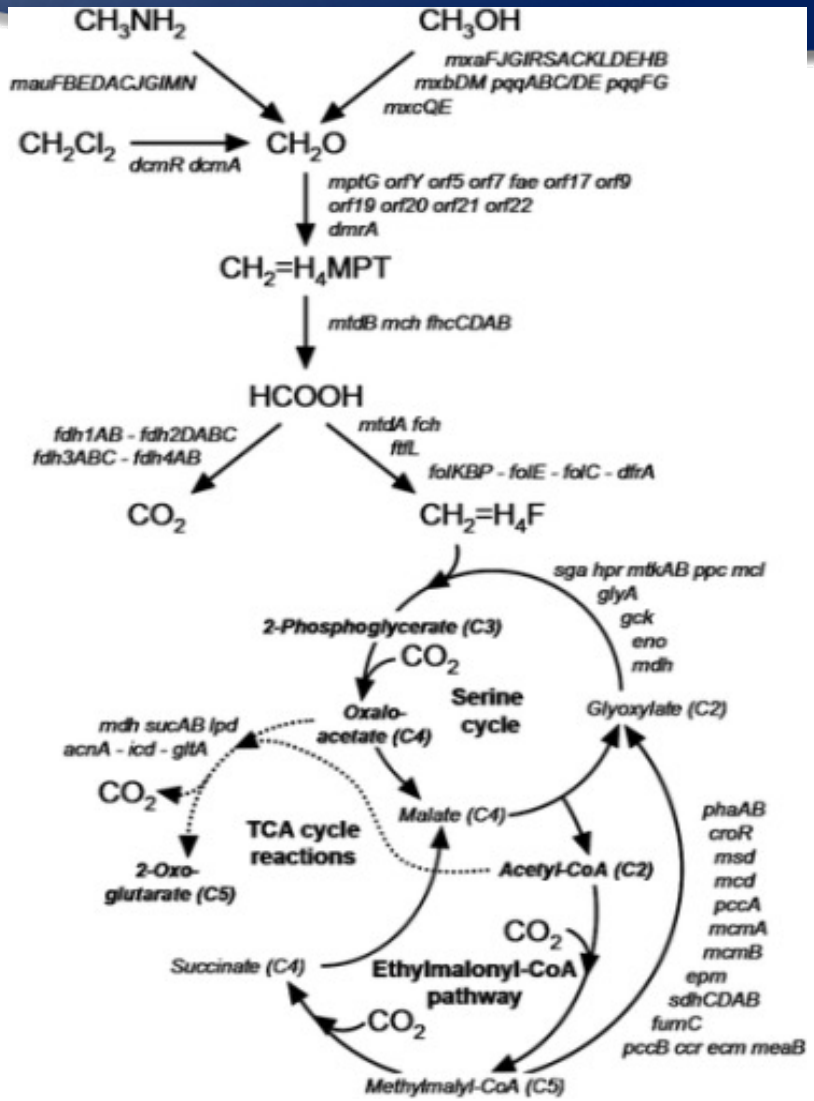
$$CH_4 + O_2 + NAD(P)H + H^+ \rightarrow CH_3OH + NAD(P)^+ + H_2O$$

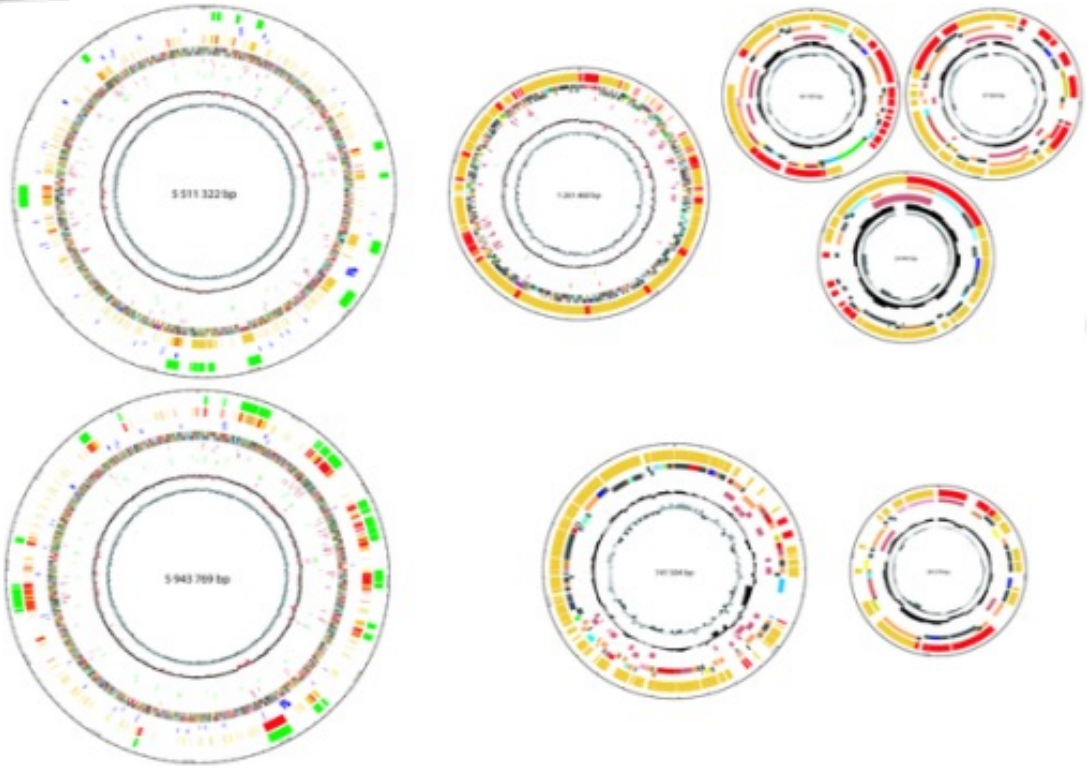
Methylobacterium adhaesivum



- Alpha-Proteobacterium
- Gram-negativ
- fakultativ methylotroph
- wächst auf Methylamin, Methanol
  C_2-, C_3- und C_4- Verbindungen
- Findet sich auf Pflanzenoberflächen, Böden, z.T. gibt es auch Endosymbionten; ebenfalls Teil der Mundflora

andere Gattungen: *Methylosinus*, *Methylocystis*, *Methanomonas*, *Methylomonas*, *Methanobacter*, *Methylococcus*



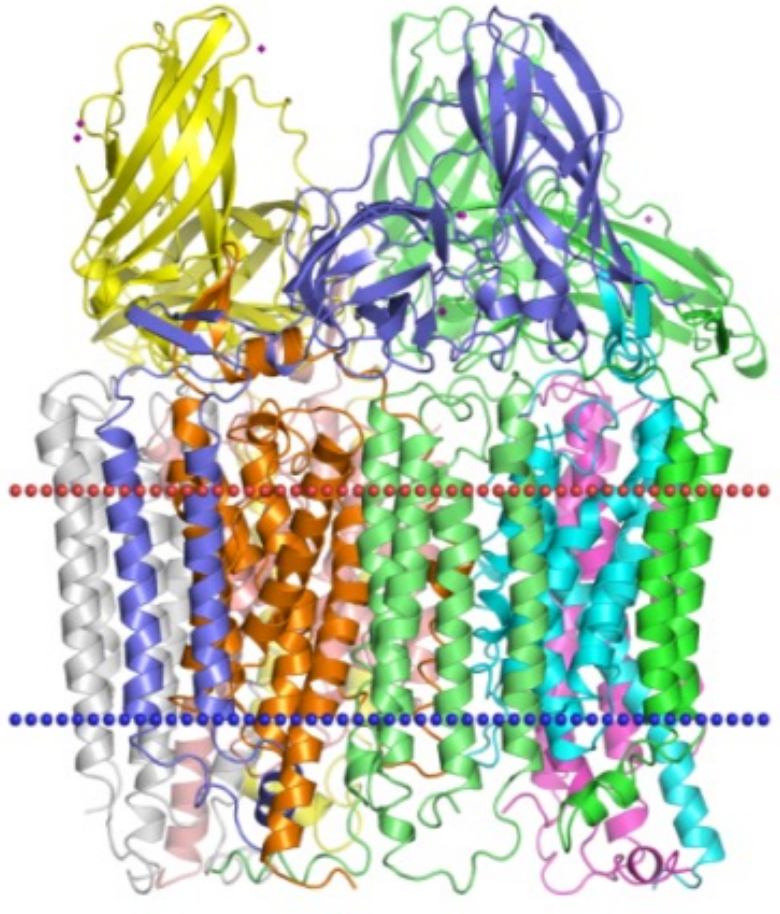


Schematic representation of the 8 circular replicons in the genomes of *Methylobacterium extorquens* strains AM1 (top) and DM4 (bottom)

Vuilleumier S, Chistoserdova L, Lee M-C, Bringel F, et al. (2009) Methylobacterium Genome Sequences: A Reference Blueprint to Investigate Microbial Metabolism of C1 Compounds from Natural and Industrial Sources. PLoS ONE 4(5): e5584. doi:10.1371/journal.pone.0005584
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0005584

Methanmonoxigenase:
Oxidoreductase (EC 1.14.13.25)

- drei UE
- 7 Transmebranhelices
- ABC Homotrimer
- die Struktur zeigt, dass das Enzym selbst als Dimer aktiv ist
- membranintegriert
- sehr ähnlich zur Ammoniummonoxygenase
- Zudem Cu2+ und Zn2+
- Substratspektrum recht weit: Methan, Ammonium, halogenierte C-Verbindungen und Aromaten



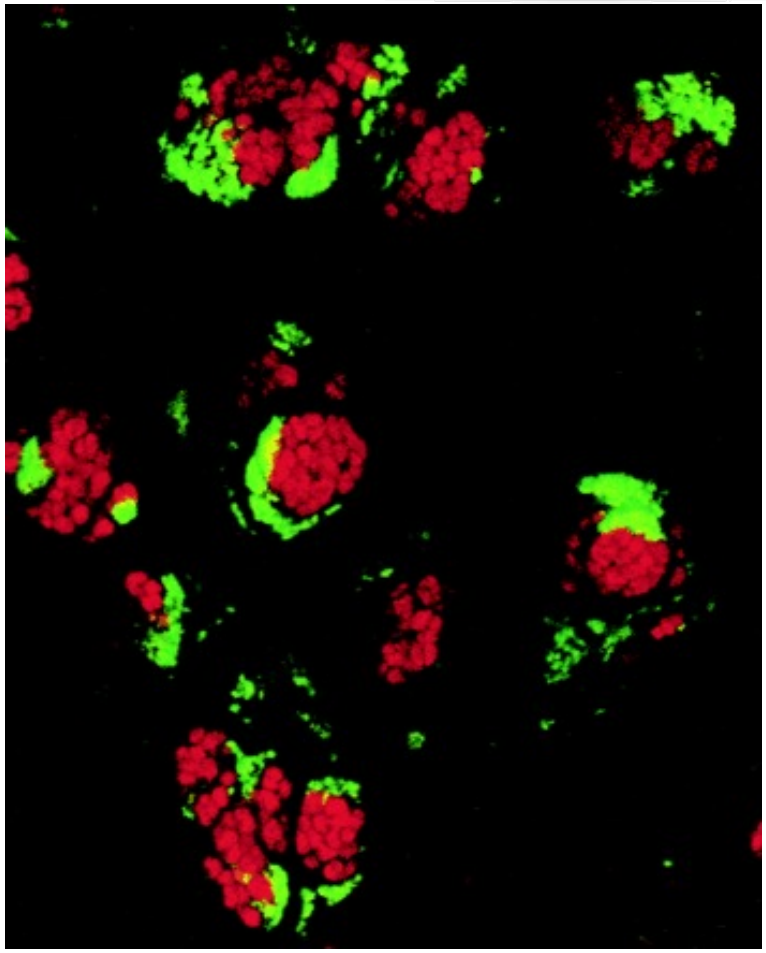
Anaerobe Oxidation von Methan (**AOM**)

- findet in marinen Sedimenten statt
- daran beteiligt sind sogenannte "synthrophe Konsortien" aus Archaeen und Sulfat-reduzierenden Bakterien

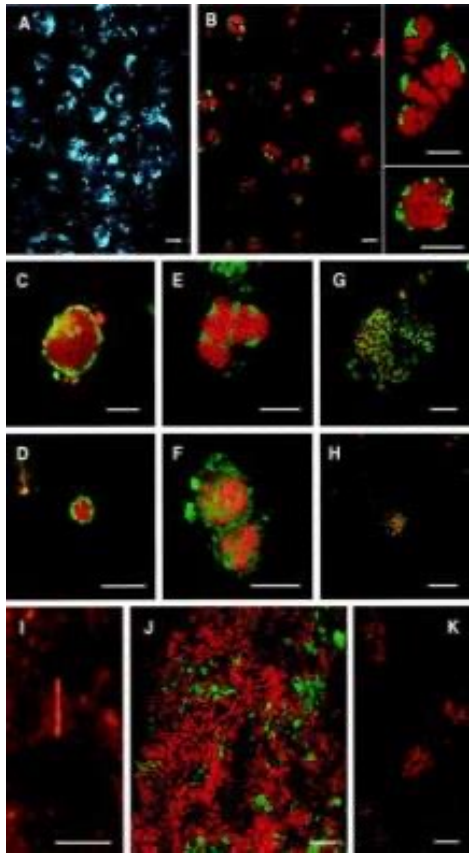
$$CH_4 + SO_4^{2-} \rightarrow HS^- + HCO_3^- + H_2O$$

AOM ist wichtiger als die aerobe Oxidation von Methan!

Archaea werden als ANME 1-3 bezeichnet, nicht kultivierbar!



American Society for Microbiology; Knittel et al., 2005
Appl. Environ. Microbiol. 2005;71:467-479

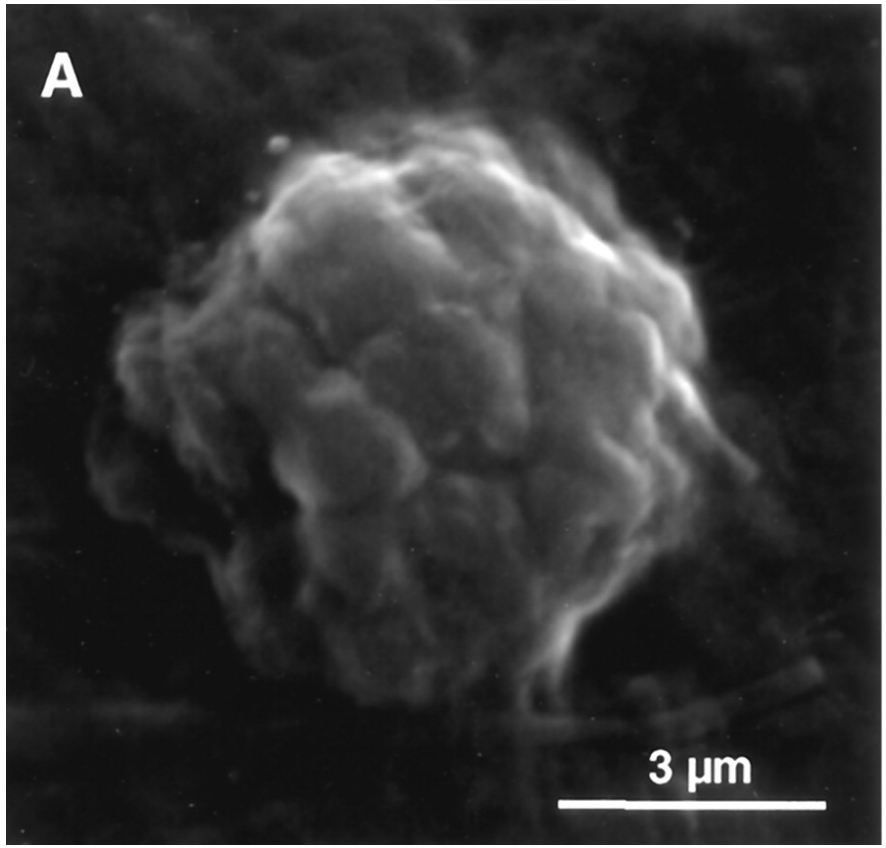


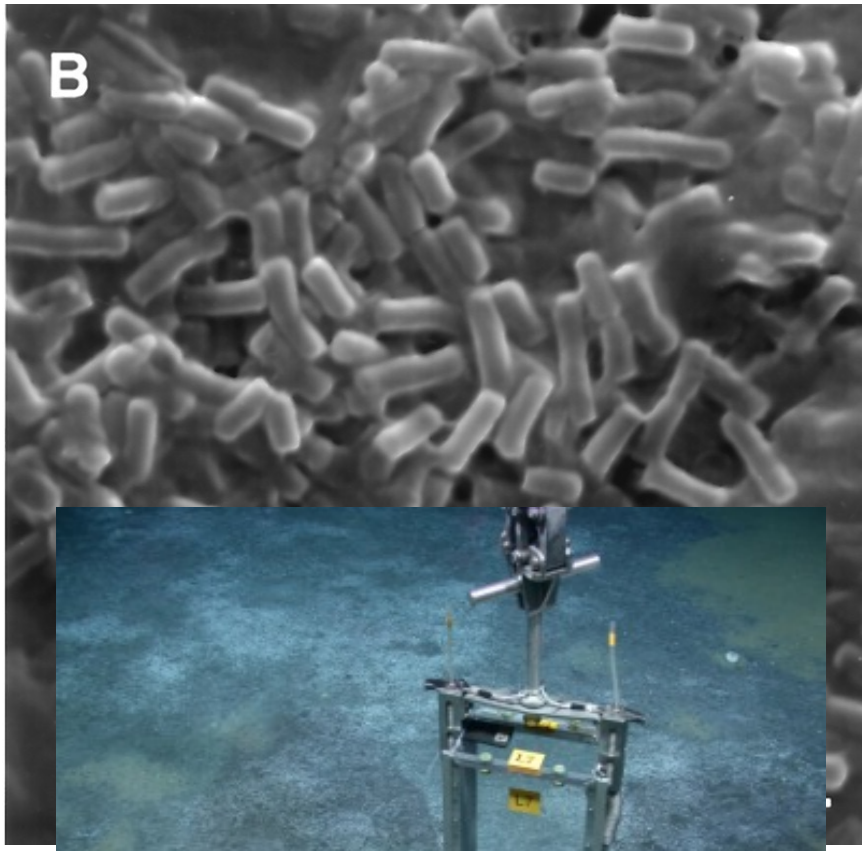
Bar 10µM

ANME
(rote Fluoreszenz)

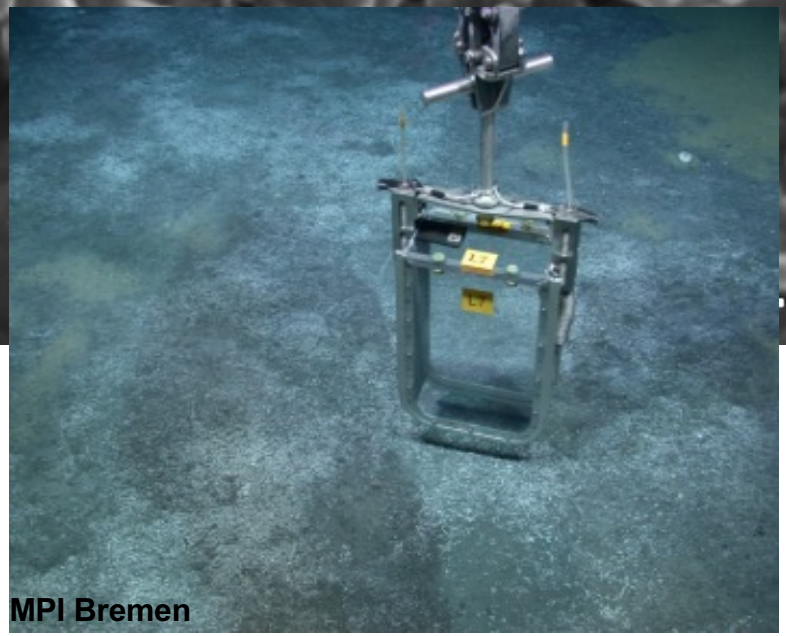
und

Sulfatreduzierer
Desulfosarcina/
Desulfococcus Gruppe
(grüne Fluoreszenz)
aus dem Schwarzen Meer
(2005)





Knittel K et al. Appl. Environ. Microbiol. 2005;71:467-479



LETTER

doi:10.1038/nature10663

Structure of a methyl-coenzyme M reductase from Black Sea mats that oxidize methane anaerobically

Seigo Shima[1,2], Martin Krueger[3], Tobias Weinert[4], Ulrike Demmer[4], Jörg Kahnt[1], Rudolf K. Thauer[1] & Ulrich Ermler[4]

The anaerobic oxidation of methane (AOM) with sulphate, an area currently generating great interest in microbiology, is accomplished by consortia of methanotrophic archaea (ANME) and sulphate-reducing bacteria[1,2]. The enzyme activating methane in methanotrophic archaea has tentatively been identified as a homologue of methyl-coenzyme M reductase (MCR) that catalyses the methane-forming step in methanogenic archaea[3,4]. Here we report an X-ray structure of the 280 kDa heterohexameric ANME-1 MCR complex. It was crystallized uniquely from a protein ensemble purified from consortia of microorganisms collected with a submersible from a Black Sea mat catalysing AOM with sulphate[4]. Crystals grown from the heterogeneous sample diffract to 2.1 Å resolution and consist of a single ANME-1 MCR population, demonstrating the strong selective power of crystallization. The structure revealed ANME-1 MCR in complex with coenzyme M and coenzyme B, indicating the same substrates for MCR from methanotrophic and methanogenic archaea. Differences between the highly similar structures of ANME-1 MCR and methanogenic MCR include a F_{430} modification, a cysteine-rich patch and an altered post-translational amino acid modification pattern, which may tune the enzymes for their functions in different biologi

Large amounts (u
methane, are oxidize
sortia of methanotro
and sulphate-reducir
atmosphere[1].

$CH_4 + SO_4^{2-} \rightarrow$ I

methane to CH_3-S-CoM at specific rates sufficient to account for those observed *in vivo* for AOM[3].

Despite these well-founded arguments for MCR as the methane-oxidizing enzyme, its catalytic activity and its coenzyme specificity are not measurable as the purified enzyme is isolated in an inactive form. All attempts to reactivate the enzyme, as measured by CH_3-S-CoM reduction to methane with HS-CoB, have failed so far. Coenzyme specificity of ANME-1 MCR might therefore differ from that of methanogenic MCR, which is specific for HS-CoM and HS-CoB, and indeed, recent data suggest that methanethiol rather than CH_3-S-CoM might serve as an intermediate in AOM with sulphate[13]. Moreover, ANME-1 MCR shows significant differences compared to methanogenic MCR regarding the prosthetic group F_{430} (ref. 12), the accumulation of cysteines near F_{430} (ref. 12) and post-translational modifications[14].

A structural characterization of ANME-1 MCR by X-ray analysis would provide important contributions to the molecular understanding of AOM; however, its determination has to involve novel methods because methanotrophic archaea cannot be grown in pure cultures and a recombinant production of MCR has not yet been possible because of its unusual F_{430} cofactor and post-translational modifications[14]

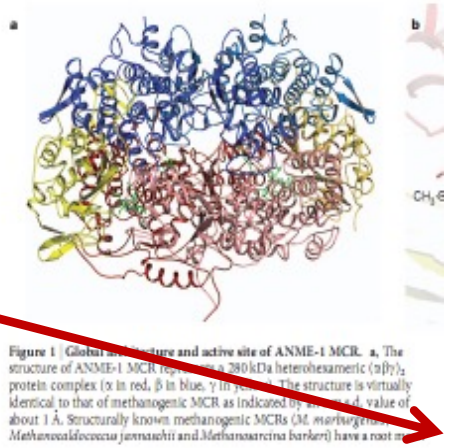
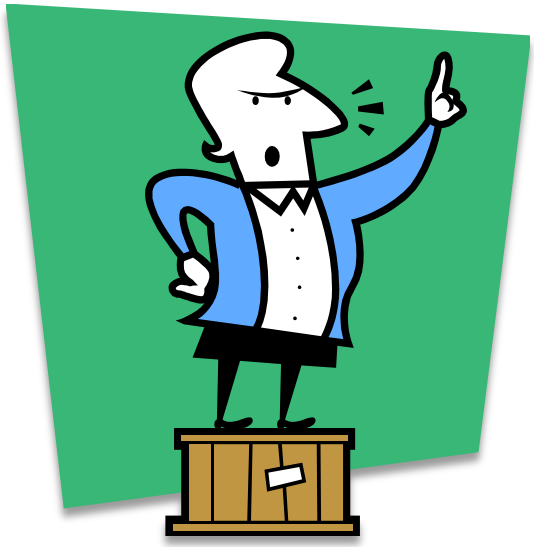


Figure 1 | Global structure and active site of ANME-1 MCR. a, The structure of ANME-1 MCR represents a 280 kDa heterohexameric $(\alpha\beta\gamma)_2$ protein complex (α in red, β in blue, γ in yellow). The structure is virtually identical to that of methanogenic MCR as indicated by the r.m.s.d. value of about 1 Å. Structurally known methanogenic MCRs (*M. marburgensis*, *Methanocaldococcus jannaschii* and *Methanosarcina barkeri*) have a root m

structure of the 280 kDa heterohexameric ANME-1 MCR complex. It was crystallized uniquely from a protein ensemble purified from consortia of microorganisms collected with a submersible from a Black Sea mat catalysing AOM with sulphate[4]. Crystals grown from

Thema der Vorlesung



Kohlenstoffkreislauf

Acetogenese

Homoacetogene MO

Vorkommen:
Acetogene MO kommen im Schlamm von Gewässern, Faultürmen
von Kläranlagen vor und spielen eine wichtige Rolle in der anaeroben Nahrungskette.

Produkte:
Acetat als Endprodukt. Grundlage dazu sind z.B. CO_2 und H_2

→ Homoacteogenese, **Acetyl-CoA-Weg**

$$4\ H_2 + 2\ CO_2 \rightarrow CH_3\text{-}COOH\ (\Delta G^{0'} = -111\ kJ/mol$$

Es gibt aber auch: → Essigsäuregärung

Acetat, CO_2

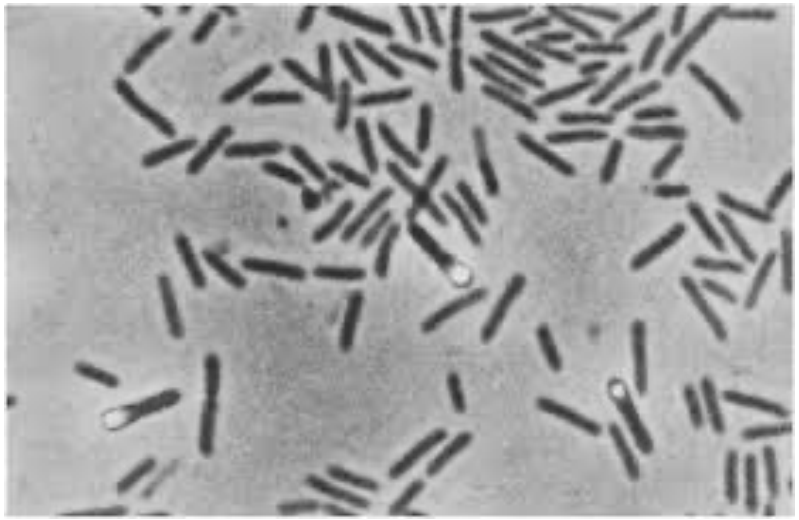
Acetat, CO_2, H_2

→ nur, wenn H_2 gleichzeitig durch H_2-verwertende Bakterien (z.B. methanogene MO) verbraucht wird. Syntrophe Interaktion

Wood-Ljungdahl-Pathway

Homo-Acetogene MO:
Clostridium aceticum,
Moorella (Clostridium) thermoaceticum
Moorella thermoautotrophica
Clostridium ljungdahlii
Sporomusa malonica

Aber auch Archaeen
Archaeoglobus fulgidus

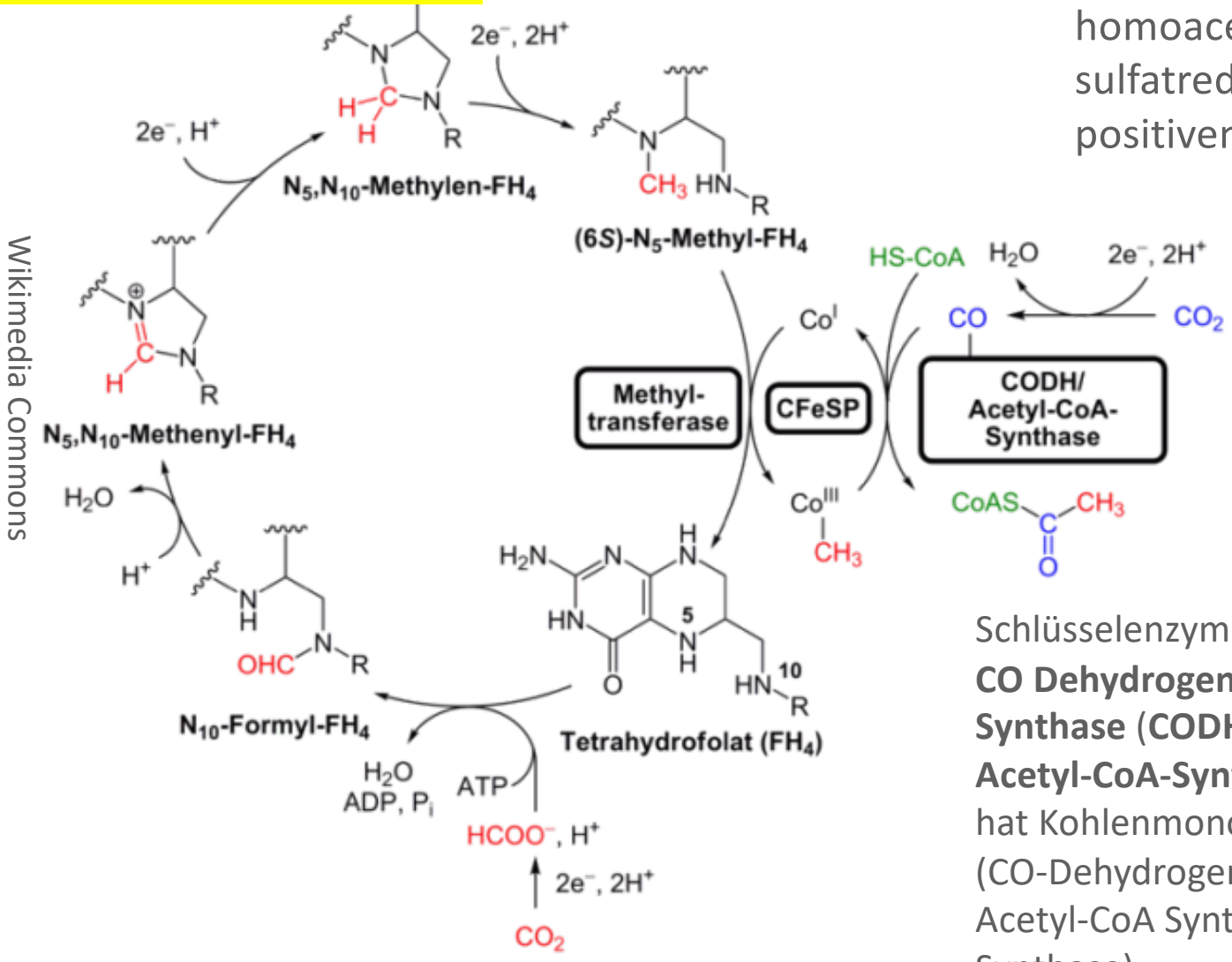


Morella (Clostridium) thermoaceticum
Semantic.scholar.org

$4\ H_2 + 2\ CO_2 \rightarrow CH_3\text{-}COOH\ (\Delta G^{0'} = -111\ kJ/mol)$

Enzymatik etwas unterschiedlich bei Archaea vs. Bacteria

Wood-Ljungdahl-Pathway



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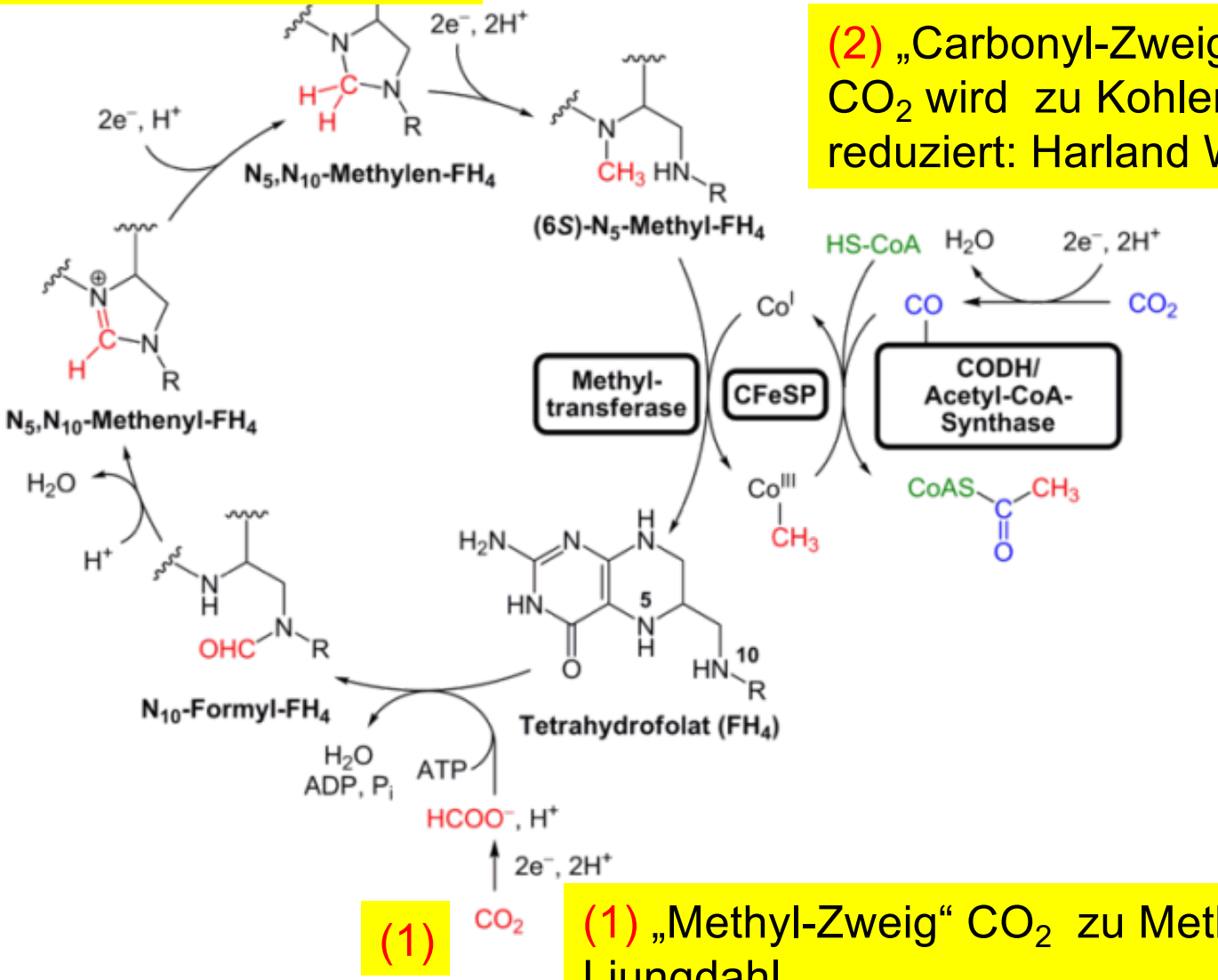
Acetyl-CoA-Weg bei allen homoacetogenen und sulfatreduzierenden Gram – positiven Bakterien.

Schlüsselenzyme:
CO Dehydrogenase/Acetyl-CoA-Synthase (**CODH/ACS** bzw **Acetyl-CoA-Synthase-Komplex**),
hat Kohlenmonoxiddehydrogenase-Aktivität (CO-Dehydrogenase) &
Acetyl-CoA Synthese Funktion (Acetyl-CoA-Synthase)

Wood-Ljungdahl-Pathway



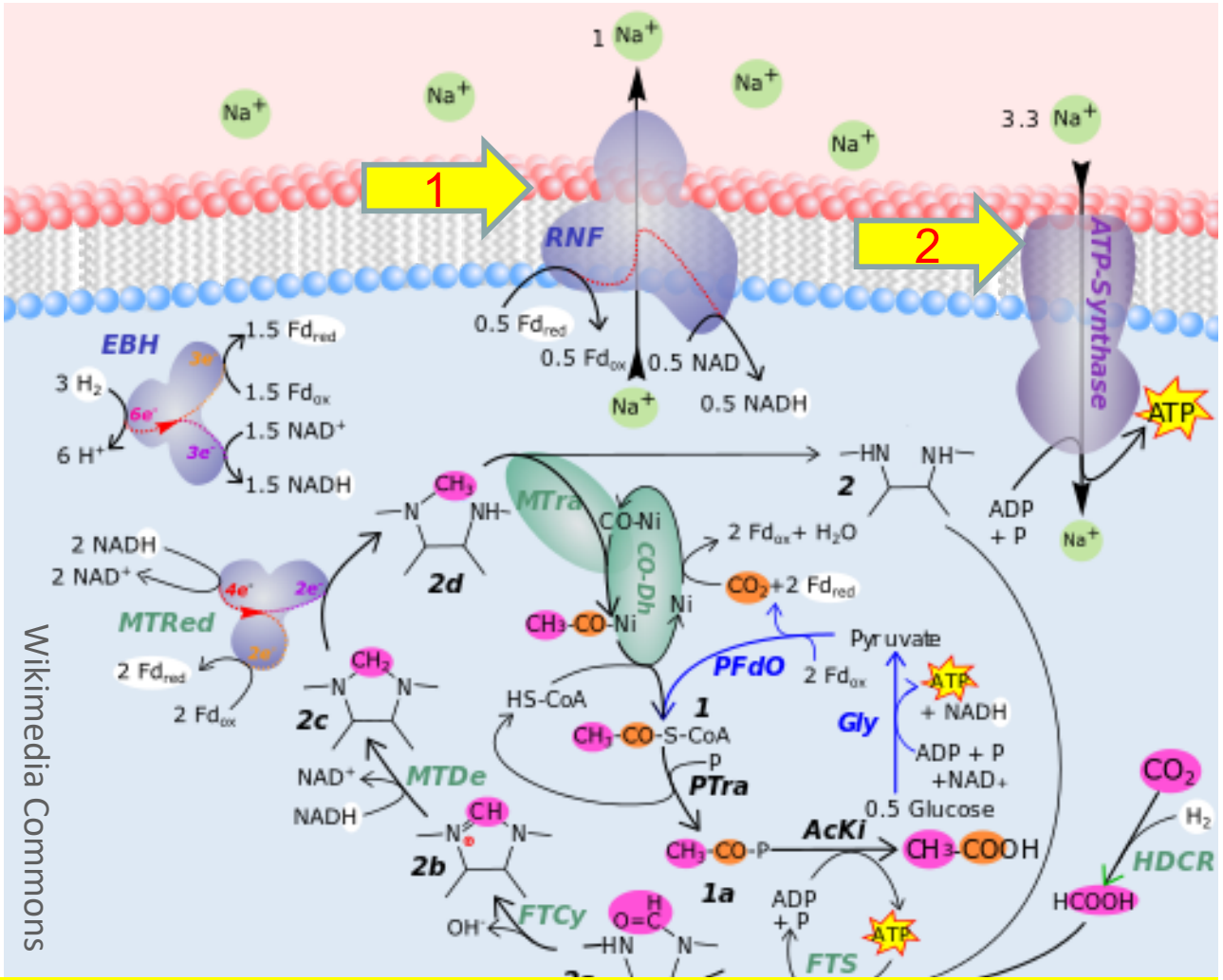
(2) „Carbonyl-Zweig“, das zweite Molekül CO_2 wird zu Kohlenstoffmonoxid, CO reduziert: Harland Wood

(2)

(1)

(1) „Methyl-Zweig“ CO_2 zu Methylrest, CH_3 : Lars Ljungdahl.

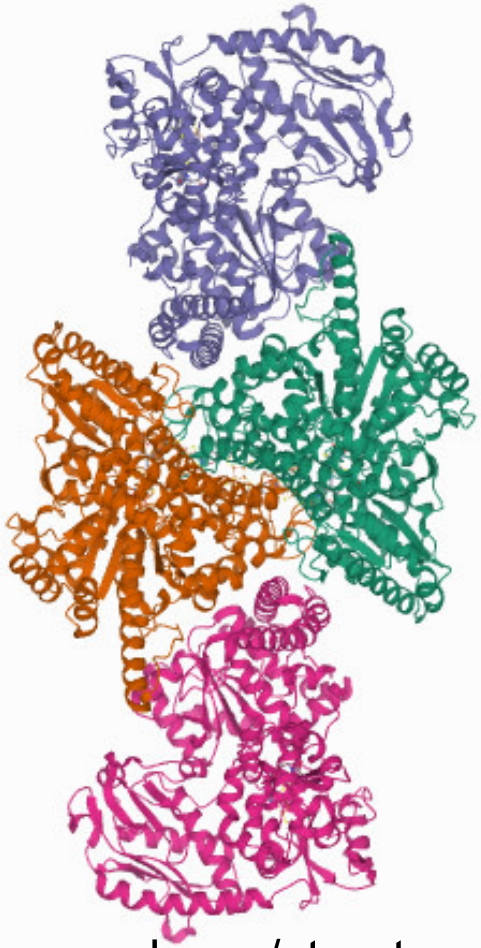
1 Acetyl-Coenzym A, ***1a*** Acetylphosphat, ***2*** Tetrahydrofolat (THF), ***2a*** Formyl-THF, ***2b***Methenyl-THF, ***2c*** Methylen-THF, ***2d*** Methyl-THF, ***AcKi*** Acetatkinase, ***CO-Dh*** CO-Dehydrogenase / Acetyl-CoA-Synthase, ***EBH*** Elektronenbifurkierende Hydrogenase, ***Fdox***Ferredoxin, ***Fdred*** Ferredoxin $^{2-}$, ***FTCy*** Formyl-THF-Cyclohydrolase, ***FTS*** Formyl-THF-Synthetase, ***Gly*** Glycolyse, ***HDCR*** H_2-abhängige CO_2 - Reductase, ***MTDe*** Methylene-THF-Dehydrogenase, ***Mtra*** Methyltransferase, ***MTRed*** Methylene-THF-Reductase, ***PFdO***Pyruvat:Fd-Oxidoreductase, ***Ptra*** Phosphotransacetylase, ***RNF*** Ferredoxin:NAD-Oxidoreductase (Rnf-Komplex).



Energiegewinnung über (1) Na-abhängige RNF/Ech NADH-Oxred und (2) ATP-Synthase.

Bifunktionales Schlüsselenzym des Wood-Ljungdal-Wegs: : CARBON MONOXIDE DEHYDROGENASE/ACETYL-COA SYNTHASE(CODH/ACS)

MOORELLA THERMOACETICA



https://www.rcsb.org/structure/1mjg